

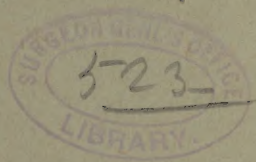
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On Some of the Tests for Quinine.

BY

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The recognition of quinine by chemical tests, when present in notable quantity, is usually a matter of great ease. Under certain conditions, however, as in its extraction from complex organic mixtures or from the tissues, it may be recovered only in minute quantity, and its presence may then not be so readily determined, at least by purely chemical tests. Among the strictly chemical tests for this substance, the *thalleioquin* reaction as it is termed, is one of the most characteristic, and at the same time one of the most delicate. This test, however, requires caution in its application, since it may give a negative result even in the presence of quinine in considerable quantity.

I. THALLEIOQUIN TEST.

This test was first proposed by Prof. M. Andre, of Mentz, in 1835,¹ and consists in the production of a bright emerald green coloration when a solution of a salt of quinine is treated with chlorine water, followed by the addition of a little aqua ammonia. M. Andre observed that this order of the application of the reagents was necessary for the production of the green color.

R. Brands, in 1839,² more fully examined this reaction and found that the production of the green color was dependent upon the presence of the substances employed in certain proportions, otherwise it did not appear. He found that the chlorine caused a

¹ See this JOURNAL, viii, 208.

² See *Ibid.*, xi, 36.

Ammon. y. over. thalleioquin. Dec. 1839



decomposition of the quinine which varied with the amount of chlorine employed, and thus determined whether a green precipitate would be produced, or the liquid simply acquire a green color or become yellow.

The green precipitate thus produced was found to have a bitter taste similar to that of quinine; was insoluble in cold water and only sparingly soluble in boiling water; insoluble in ether, but readily soluble in alcohol, and readily soluble in diluted acids forming red solutions, from which it was reprecipitated of a green color on neutralizing the solution with ammonia.

In 1853, A. Vogel¹ proposed to modify this test by treating the quinine solution after addition of chlorine water, with a solution of potassium ferrocyanide and then adding a few drops of ammonia, when the mixture would assume a deep *red* color, the green color not appearing.

Professor Flückiger, in 1861, confirmed the observation of Vogel and found that the same red coloration might be produced by substituting potassium ferricyanide for the ferrocyanide.

More recently, Chas. F. Zeller² has examined this test in regard to the production of a green coloration under the action of chlorine and ammonia, and confirmed the observations of Brands, namely: that the results were influenced by the relative proportion of the substances employed.

It being thus shown that this test was much influenced by the relative proportions of quinine and chlorine present, the following investigations were made for the purpose of more definitely determining the range within which the green coloration would manifest itself in different quantities of the same solution, and in solutions of different degrees of dilution.

The quinine was employed in solution both as sulphate and hydrochloride, the 1-100th solutions being prepared by dissolving, by the aid of just sufficient of the diluted acid to effect solution, 1 gramme of the pure alkaloid in 100 c.c. of water. The more dilute solutions were prepared from the 1-100th solutions, by the required dilution with water.

The chlorine water consisted of a saturated aqueous solution of

¹ This JOURNAL, 1853, 516.

² See *Ibid.*, 1880, 385.

the washed gas. This solution, for uniformity of results, should be freshly prepared and preserved from the action of light. When this reagent was used in the form of drops, these were delivered from a pipette delivering on an average twenty-five drops per cubic centimetre. The ammonia employed had a density of .950.

A. 1-100th solution of quinine.

1. 5 c.c. of this solution (= 50 mgms. quinine) yields with:

(a) 0.1 c.c. of chlorine water: a colorless solution which, on the addition of a drop of ammonia, yields a copious white precipitate (of quinine), and after a little time, the mixture acquires a rose-red color; on the addition of a second drop of ammonia, the mixture becomes colorless, then slowly assumes a greenish hue which becomes well marked.

(b) 0.5 c.c. of chlorine water, followed by a drop of ammonia, yields a copious white precipitate, and the mixture quickly assumes a green color, then becomes dark rose-red and finally green.

(c) 1.0 c.c. chlorine water and a drop of ammonia: an immediate green color, which quickly changes to dark red, then to purple or blue.

2. 1 c.c. of a 1-100th solution (= 10 mgms. quinine) yields with:

(a) One drop chlorine water and then a drop of ammonia: a copious white precipitate, which quickly assumes a green color.

(b) 0.5 c.c. chlorine water and a drop of ammonia: an intense green coloration.

(c) 1.0 c.c. chlorine water and one drop of ammonia: a bright green coloration, which becomes darker in color and finally nearly black.

3. 0.1 c.c. of a 1-100th solution (= 1 mgm. quinine) in a very small, narrow test tube, yields with:

(a) One drop of chlorine water and a drop of ammonia: a copious green precipitate.

(b) Two drops chlorine water and a drop of ammonia: a bright green coloration.

(c) Three drops chlorine water and a drop of ammonia: no precipitate, but the mixture immediately acquires a bright green color, which quickly darkens.

B. 1-1000th solution of quinine.

1. 5 c.c. of the solution (= 5 mgms. quinine) yields with :
 - (a) One drop chlorine water and a drop of ammonia : a white precipitate (quinine) which quickly assumes a greenish hue, slowly changing to well-marked bluish green.
 - (b) 0.1 c.c. chlorine water and one drop of ammonia : the precipitate quickly acquires a strong, bluish-green color.
 - (c) 0.5 c.c. chlorine water and a drop of ammonia : an intense, emerald-green coloration.
 - (d) 1.0 c.c. chlorine water and one drop of ammonia, no precipitate, but a strong yellow coloration, which soon changes to deep purple.
 - (e) 2.0 c.c. chlorine water and a drop of ammonia, a colorless mixture ; on further addition of ammonia, a dirty-brown coloration may be produced.
2. 1 c.c. of a 1-1000th quinine solution, yields with one drop of ammonia, after addition of :
 - (a) One drop chlorine water : a green turbid mixture.
 - (b) 0.1 c.c. chlorine water : a bright green coloration.
 - (c) 0.5 c.c. of the chlorine reagent : a pale green coloration.
 - (d) 1.0 c.c. chlorine water : a slightly yellow mixture.
3. 0.1 c.c. of a 1-1000th solution, under like conditions, yields with :
 - (a) One drop chlorine water : a very bright green coloration.
 - (b) Two drops of the chlorine reagent : a less intense green coloration.

C. 1-5000th solution of quinine.

1. 5 c.c. of the solution = (1 mg. quinine) yields with a drop of ammonia, after addition of :
 - (a) One drop chlorine water : a colorless solution, which, after a time, may acquire a greenish hue.
 - (b) 0.2 c.c. chlorine water : a fine, emerald green solution.
 - (c) 0.5 c.c. chlorine water : a well-marked green coloration, quickly discharged on shaking the mixture.

(d) 1.0 c.c. of chlorine reagent: no coloration, but, after a time, the mixture assumes a yellow hue.

2. 1 c.c. of a 1-5000th solution, with one drop of ammonia after adding:

(a) One drop chlorine water: a fine, green coloration, changing to bluish-green.

(b) 0.1 c.c. chlorine water: a pale green coloration.

(c) 0.5 c.c. chlorine reagent: a light green color, quickly changing to a faintly yellow.

3. 0.1 c.c. of 1-5000th solution, with one drop ammonia, after adding:

(a) One drop chlorine water: a well-marked green coloration.

(b) Two drops chlorine water: only a faintly yellowish color.

D. 1-10000th solution of quinine.

1. 5 c.c. of the solution (= 0.5 mg. quinine) yields with one drop of ammonia, after addition of:

(a) One drop chlorine water: the mixture slowly acquires a greenish hue.

(b) Two drops chlorine water: a strongly marked green coloration.

(c) 0.2 c.c. of chlorine water: a colorless mixture.

2. 1 c.c. of a 1-10000th solution, yields with:

(a) One drop chlorine water and one drop ammonia: a marked green coloration.

(b) Two drops chlorine water and a drop of ammonia: the mixture may present a greenish hue, which quickly changes to yellow.

From 0.1 c.c. of a 1-10000th quinine solution, a greenish coloration may be obtained by employing a drop of a much-diluted solution of chlorine.

From 5 c.c. of a 1-20000th solution of quinine, no green coloration was obtained, even on employing a diluted solution of chlorine.

In 1872, Prof. Flückiger¹ proposed to substitute *bromine* for chlorine in this test, and stated that its reaction was much more

¹Neues Jahr. f. Pharm., 1872. 139.

delicate than that of chlorine, since under it a green coloration might be obtained from a 1-20000th solution of quinine, whereas, chlorine had its limit in about a 1-5000th solution.

In the following examinations a saturated aqueous solution of bromine was employed, it being prepared by agitating excess of bromine with water, and, after subsidence, decanting the clear, highly colored solution. The reagent should be freshly prepared, since it may, within twenty-four hours, especially if exposed to light, undergo a marked change. A saturated aqueous solution of the reagent contains practically 1 per cent. by volume, or 3 per cent. by weight of bromine.

A. 1-100th solution of quinine.

1. 5 c.c. of the solution yields with :

(a) One drop of bromine water : a copious yellow precipitate which quickly dissolves to a colorless solution, the addition of a drop of ammonia causes a white precipitate (quinine), which, after a time, acquires a bluish hue, then a bluish green color.

(b) Three drops bromine water then one drop ammonia, yield a precipitate which soon assumes a greenish blue color.

(c) 0.5 c.c. bromine water followed by one drop ammonia : the mixture quickly becomes bluish green, which increases its intensity.

(d) 1.0 c.c. bromine water causes a slightly yellow coloration ; the addition of 0.1 c.c. ammonia produces a white precipitate which quickly assumes a bright green color, which may change to purple.

The exact coloration produced in the above solutions depends somewhat upon the manner in which the reagents mix with the quinine solution. The best results are obtained by dropping the reagents into the quinine solution without agitation.

2. 1 c.c. of the quinine solution yields with one drop of ammonia after addition of :

(a) One drop bromine water : a white precipitate which soon assumes a green color.

(b) 0.2 c.c. bromine water : quickly a bright green solution.

(c) 0.5 c.c. bromine water : a purple precipitate, which, after a time, assumes a strong green color.

3. 0.1 c.c. of a 1-100th quinine solution, yields with one drop of bromine water and a drop of ammonia, a white precipitate which quickly assumes a green color.

B. 1-1000th solution of quinine.

1. 5 c.c. of the solution yields with one drop of ammonia, after adding :

(a) One drop bromine water : a white precipitate which soon assumes a bluish-green color.

(b) 0.1 c.c. bromine : an immediate bright green coloration.

(c) 0.3 c.c. bromine water alone causes a yellow coloration, which on addition of the ammonia, is changed to deep purple, and this may slowly change to very dark green.

2. 1 c.c. of the quinine solution with one drop of the bromine water and one drop of ammonia will yield a bright green coloration. Under the action of a slightly larger quantity of the bromine reagent, only a purple color will appear, or the mixture will remain colorless.

3. 0.1 c.c. of the quinine solution with a *minute* drop of the bromine water and one drop of ammonia, will yield a green coloration ; but if a full drop of the bromine reagent be employed, a colorless mixture will result. With a diluted solution of the bromine water (1:4), a fine green coloration may be obtained.

C. 1-5000th solution of quinine.

1. 5 c.c. of the solution with one or two drops of the bromine water and one drop ammonia, yields a good green coloration. With a slightly larger amount of the bromine reagent, the mixture remains colorless.

2. 1 c.c. of the quinine solution with one drop of bromine water and one drop ammonia, will after a time acquire a green coloration. If two drops of the bromine water be employed, the mixture remains permanently colorless.

3. 0.1 c.c. yields with a drop of the bromine reagent, a deep yellow color, which is quickly discharged to a permanently colorless solution by a drop of ammonia. Under the action of a drop of diluted bromine water (1:9), a bright green coloration may be obtained.

D. 1-10000th solution of quinine.

1. 5 c.c. of the solution with :

(a) One drop bromine water yields a colorless solution which on addition of a drop of ammonia quickly assumes a green color; this becomes deep bright green, which remains unchanged for many hours.

(b) Two drops of bromine water causes a yellow color, which is immediately discharged by a drop of ammonia, and the mixture remains colorless.

2. 1 c.c. of the quinine solution fails to yield a green coloration unless the bromine reagent be diluted.

3. From 0.1 c.c. of the quinine solution, no green coloration was obtained, even with the diluted bromine reagent.

E. 1-20000th quinine solution.

5 c.c. of this solution yields no green coloration with the undiluted bromine reagent; but under a dilution of 1:2 a well-marked green color may be obtained.

From the foregoing it is obvious that the production of a green color under the action of this test depends upon the presence of the quinine and bromine in proportion contained within rather narrow limits; otherwise the green coloration will not manifest itself. Should it be desired to apply this test to only a small and limited quantity of a suspected solution, it would be necessary, or at least advisable, to first ascertain under what conditions a similar volume of solution of quinine of known strength would give a positive reaction with a given quantity of the bromine reagent, after proper dilution of the latter if necessary.

If an aqueous solution of chlorine, instead of bromine, be employed in the thalleioquin test, a positive reaction will manifest itself through a greater range than when bromine is employed, the result being less readily affected by excess or deficiency of the reagent.

In regard to the production of a *red* coloration, by treating the quinine solution, after addition of bromine or chlorine, and before adding the ammonia, with *potassium ferrocyanide* solution, as advised by Vogel, the results were less satisfactory than the test without the use of the potassium salt. With solutions containing more than 1-1000th of the alkaloid, a green or red or other coloration may be

developed, the result depending upon the relative proportions of the reagents employed.

On treating 5 c.c. of a 1-1000th quinine solution with one drop of bromine water followed by one drop of potassium ferrocyanide solution (1:12) and a drop of ammonia, the result is about the same as without the presence of the potassium salt; that is, a white precipitate soon becoming green is produced. But, if under these conditions 0.2 c.c. of the potassium solution be employed, a portion of the mixture may be red and a portion green in color.

5 c.c. of a 1-5000th solution of the alkaloid under the action of one drop each of the reagents as above, yields a fine green coloration. If, however, to this quantity of the quinine solution one drop of bromine water be added and then 0.5 c.c. of the ferrocyanide solution, the mixture, without the addition of ammonia, immediately assumes a deep red color. If a drop of ammonia be now added, the red color quickly changes to a beautiful purple.

5 c.c. of a 1-10000th quinine solution with one drop each of the reagents, yields a fine red-purple coloration which soon changes to green.

5 c.c. of a 1-20000th solution, under like conditions, yields a fine red coloration, which quickly fades to a light yellow color.

2. HERAPATHITE TEST.

This test was first proposed by Dr. Herapath, in 1852, and consists in the formation of *quinine iodosulphate* or *Herapathite*, as it has been termed. This compound may be obtained by treating a solution of quinine in a mixture of strong acetic acid and alcohol, with an alcoholic solution of iodine. In a little time the iodosulphate separates out in the form of characteristic plates and rosette groups of crystals. By reflected light, the crystals are of a dark green color; under transmitted light they are dichroic and strongly polarize light.

The reagents may be prepared as follows: (a) Thirty volumes of strong acetic acid are mixed with ten volumes of strong alcohol and one volume of diluted sulphuric acid (1:10). (b) One part of iodine is dissolved in about twenty parts by weight of alcohol.

To apply the test, a drop of the quinine solution is evaporated to dryness, and the residue treated with a drop of the first-mentioned solution; a minute drop, or sufficient to color the liquid brownish-

yellow, of the alcoholic solution of iodine is then added. Very soon portions of the mixture will present a dark green deposit, which when examined by a moderate power (75 diam.) of the microscope will be found to consist of the crystals in question. The formation of these crystals is perfectly characteristic of quinine.

The residue from *one drop* of a 1-100th solution of quinine, in the form of sulphate, when treated with a drop of the acetic acid mixture and then sufficient of the iodine solution to impart a strong color, will yield innumerable crystals of the iodosulphate compound.

A 1-500th solution residue will generally yield an abundant deposit of the crystals.

The residue from a drop of a 1-1000th solution, if simply moistened with the acetic acid liquid and then with a minute drop of the iodine solution, may yield satisfactory results; but it requires a nice adjustment of the reagents to obtain satisfactory results from this quantity of the alkaloid.

3. FLUORESCENCE.

One of the most striking properties and at the same time the most delicate reaction of quinine at present known, is the fluorescence of solutions of its oxysalts, especially the sulphate. Normal solutions of the hydrochloride, hydrobromide, hydriodide and hydrocyanide present little or no fluorescence. A solution of the hydrochloride containing a limited excess of hydrochloric acid, may, as pointed out by Prof. R. A. Witthaus,¹ present, a well marked or even strong fluorescence, especially in dilute solutions; but this is permanently destroyed by a slightly larger quantity of the free acid.

When in solution as quinine normal acid sulphate, without excess of acid, and examined by ordinary reflected light in volumes of 50 to 100 c.c. the blue fluorescence is very intense in the 1-100th, 1-1000th, and 1-10000th solutions, and very well marked in a 1-50000th solution; but it is not apparent in a 1-100000th solution, and is only faintly marked even under a cone of condensed sunlight.

If 1 c.c. of the acid sulphate solution, placed in a small test tube, be examined it will present as follows:

(1) 1-100th solution, a very strong blue fluorescence in reflected light, which becomes intense in condensed sunlight.

¹ Researches Loomis Laboratory, 1892, 91.

(2) 1-1000th solution, a strongly marked fluorescence in reflected light; only feebly marked in direct sunlight; but intense in a cone of sunlight.

(3) 1-10000th solution, a just perceptible fluorescence in reflected light; appears colorless in sunlight; intensely fluorescent in a cone of sunlight.

(4) 1-50000th solution exhibits no marked fluorescence in either reflected or in direct sunlight, and only a faint fluorescence under a cone of sunlight. On the addition of a drop of dilute sulphuric acid, the solution presents a quite well-marked fluorescence in condensed sunlight.

(5) 1-100000th solution in condensed sunlight presents only a faint fluorescence; but on addition of a drop dilute sulphuric acid, the fluorescence is well marked.

The last-mentioned degree of dilution is by no means the limit of the fluorescence of quinine solutions, under certain conditions. Kerne, rby a specially constructed instrument, termed by him the fluoscope, was able to obtain the fluorescence beautifully marked in a solution of the alkaloid under a dilution of two million times.

It should be borne in mind that the fluorescence of quinine solutions, even of the sulphate, may be entirely prevented by the presence of chlorides, bromides and iodides, and the free acids of these salts. If to 1 c.c. of a 1-100th solution of quinine sulphate, which is strongly fluorescent, one drop of hydrochloric acid or of a solution of sodium chloride (1 : 10), be added, the fluorescence is immediately and wholly destroyed, and is not reproduced on the addition of even 0.5 c.c. of dilute sulphuric acid. The interference of bromine and iodine is as prompt and complete as that of chlorine.

According to the recent observations of MM. Sestini and Campani¹ the fluorescence of sulphuric acid solutions of quinine, especially when dilute, may also be concealed by the presence of *phenacetine*. According to these observers, this substance may also greatly interfere with the normal reaction of quinine with both chlorine and bromine in the thalleioquin test.

The property of fluorescence is possessed, although in a less degree, by some of the other cinchona alkaloids; and Dr. Bence Jones, of London, has described a substance normally present in the body, which has a similar property, and named by him *animal quinoidine*.

¹ Jour. Chem. Soc., Abs., May, 1892, 665.

Beside these substances, certain vegetable principles and extracts and the hydrocarbon oils present fluorescent properties.

4. TEST OF TASTE.

The intensely bitter taste of quinine and its salts may serve as a test of its presence. Of the ordinary preparations of quinine, the tannate is the least and the free alkaloid next least bitter; the normal sulphate is less bitter than the bisulphate, hydrobromide or hydrochloride.¹

The taste of the acid sulphate is very distinct and strongly marked in a single drop of a 1-10000th solution; and is still distinct in the same quantity of a 1-20000th solution; but, according to several persons, is not perceived in a drop of a 1-50000th solution.

In the very elaborate investigations of Dr. G. Kerner² on the absorption and elimination of quinine, he found that when taken into the stomach in 0.5 gram (7.5 grains) doses, it appeared in the urine in fifteen minutes thereafter; and that one-half of the total quantity taken was thus eliminated in six hours; and one-fourth in the following six hours; but that a trace might still be present in the urine at the end of seventy-eight hours.

Of the quinine thus eliminated Dr. Kerner found that a large portion had undergone a material change, in that while it still possessed fluorescent properties, responded to the reaction with chlorine and ammonia, and with chlorine and potassium ferrocyanide, it no longer had a bitter taste, it now being tasteless. By experiments he found that under the limited oxydation of quinine by potassium permanganate, a substance was produced which was tasteless and corresponded in every respect to this eliminated quinine. On analysis this oxydation product was found to be *dihydroxyl quinine*, having the composition $C_{20}H_{24}N_2O_2 \cdot 2OH$. From these results Dr. Kerner concluded that it was under this form that the alkaloid, in part at least, appeared in its tasteless condition in the urine.

If then, in the absorption of quinine or under oxydation, this change to the tasteless variety may take place, it is easy to understand that in the extraction of the alkaloid from the urine or the tissues, this important corroborative test might fail, even when the chemical tests indicated its presence. At present we have little or

¹ Prescott Organic Analysis, p. 127.

² Archiv für Physiologie, ii, 1869, pp. 200-243; iii, 1870, 93-165.

no experience in regard to the recovery of quinine from the body, long periods after death.

That the alkaloids in general may undergo a slow and gradual change in the presence of decomposing animal matter seems to be fully established by the investigations of Profs. Buchner, Gorup-Besanez and others¹, as well as our own observations, in the case of strychnine, which, under the conditions stated, after a time, loses its property of responding to the color and certain other chemical tests, but still retains its bitter taste and the property of producing tetanic convulsions in frogs.

So also, Dr. Pellacani² on mixing a definite quantity of *curarine* with a given quantity of fresh blood and allowing the mixture to putrify under favorable conditions of temperature, found after some months that the alkaloid no longer responded to the physiological test, although it still retained its chemical properties, except with the sulphuric acid test.

From our own experience we are strongly led to believe that when morphine and strychnine are taken in moderate toxic quantity, that portion of the alkaloid which is carried to the tissues by the ordinary process of absorption, entirely loses its property of crystallizing, even when present in quantity sufficient to promptly respond to chemical tests.

When the morphine or strychnine is taken in excessive quantity, a portion seems to be distributed to the organs by simple exosmotic diffusion, and this may readily be recovered in the crystalline state. In a case in which 1.68 grams (about 26 grains) of strychnine were administered subcutaneously to a dog during a period of four hours, in divided doses, so as to keep the animal paralyzed, 56 mgs. of well-crystallized strychnine was recovered from the urine, 26 mgs. from the liver, and 14 mgs. from the blood.

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¹ *Ann. d' Hyg.*, 1881, 385.

² *Rivista Sper. Med. Legal*, xiii, 2, p. 237.

